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Adherence of Candida albicans to Flexible Denture Base Material

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الخلاصة

الأهداف: تهدف الدراسة الى تقييم التصاق المبيضات البيضاء على مواد قاعدة طقم الأسنان المرن (الفالبلاست) و الراتنج الاكريلي المتصلب حراريا بوجود و غياب اللعاب. **المواد وطرائق البحث** : صنعت 28 عينة مربعة (10×10×3م م) من مادة الفالبلاست و الراتنج الاكريلي المتصلب حراريا وحسب تعليمات المصنع وأبقيت بدون تنعيم وصقل. تم عزل المبيضات البيضاء من المرضى الذين لديهم التهاب الفم المصاحب للطقم ، (14)عينة (7 لكل مادة) لم تغطى باللعاب (عينة سيطرة) وغمرت في معلق الخميرة (10^7 خلية \مل) وحضنت في درجة حرارة الغرفة لمدة ساعة واحدة ثم غسلت بالفوسفيت بفرسلاين ثم صبغت بصبغة الكريستال فيوليت. اما (14) عينة المتبقية (7 لكل مادة) فقد تم غمرها باللعاب وعوملت بنفس الطريقة السابقة . تم إحصاء عدد الخمائر الملتصقة في 84 حقل مجهري(0.25 م م^2 \حقل) وعبر عن النتائج بعدد الخلايا لكل مليمتر مربع . **النتائج**: عند المقارنة بين مادتي قاعدة الطقم، فان التصاق المبيضات على الراتنج الاكريلي (170 خلية خميرة \م م^2) أكثر مما هوعلى الفالبلاست (126 خلية خميرة \م م^2)، إن معدل التصاق المبيضات على عينات الراتنج الاكريلي غير المغطاة و المغطاة باللعاب (208،132 خلية خميرة \م م^2) اكبر من معدله على عينات الفالبلاست غير المغطاة و المغطاة باللعاب (177،175خلية خميرة \م م^2) بالتتالي، بعد تغطية المواد باللعاب أصبح اختزال عدد الخمائر كبيرا في كلا المادتين . **الاستنتاجات**: كان الاختزال كبيرا في عدد الخمائر الملتصقة على الفالبلاست وخاصة العينات المغطاة باللعاب مقارنة بالراتنج الاكريلي.

ABSTRACT

Aims: Assessment of Candida albicans adherence to flexible denture base material (Valplast) and conventional heat polymerized acrylic resin in the presence and absence of saliva**. Materials and Methods**: A total of 28 square specimens (10 mm x10 mm x3mm) were constructed from Valplast and acrylic denture base materials according to manufacturers' instructions and kept without finishing or polishing. C. albicans was isolated from patients with denture stomatitis. Fourteen specimens (7 of each material) were not coated with human saliva (control), deposited in yeast suspension (10^7) yeast cells/ml, incubated for 1hr at room temperature and washed with phosphate buffer saline and then stained with crystal violet. The remaining 14 specimens (7 of each material) were coated with saliva and treated as previously described. Adherent yeast cells in 84 fields of view (0.25mm^2/field) of materials were enumerated. The results were expressed as yeast cells/mm^2 of material. **Results:** In comparison between the two denture base materials, Candida adherence to acrylic resin (170 yeast cells/mm^2) is greater than Valplast (126 yeast cells/mm^2). Both saliva uncoated and coated acrylic samples have higher means of Candida adherence (208, 132 cells/mm^2) than saliva uncoated and coated Valplast samples (175, 77 cells/mm^2) respectively. High significant reduction in yeast counts was seen in both materials after saliva coating. **Conclusions:** Great reductions in yeast counts were determined in Valplast material specially in saliva coated specimens when compared with acrylic resin.

Key Words: Candida albicans adherence, flexible denture base material, Valplast

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INTRODUCTION

The ability of Candida species to adhere to oral and plastic surfaces is crucial in pathogenesis. Such adherence enables the microorganism to withstand the mechanical washing action of saliva and it is

a prerequisite for successful colonization.[1] According to several studies conducted in universities and hospitals, 65% of denture wearers suffer from problems caused by Candida albicans (which is the most adherent Candida species). This condition can lead to denture intolerance.[1, 2] Candida adherence to oral epithelium,[2, 3] soft denture lining materials,[4-6] and denture base materials,[5-7] has been studied intensively. To date, up to 95% dental prostheses are composed of polymethylmethacrylate (PMMA).[8] For instance, Candida adhesion onto PMMA- based resins is a common source of oral cavity infection and stomatitis.[9]

A thermoplastic material of dental prostheses, Valplast (Valplast Int-Corp-USA) was first introduced to dentistry in the 1950s. It is a polyamide (nylon plastic). This material is an injectable nylon-based resin to create semi-translucent flexible denture base.[10-11] Valplast satisfies both dentist and patients as an ideal alternative for full and partial acrylic dentures in a variety of circumstances.[12-13] Valplast denture is a strong, flexible, durable lightweighted, virtually invisible, comfortable nylon plastic that makes wearing dentures so pleasant.[12-14] Despite continuing improvement of this flexible Valplast material, candidal adherence of this material has not been yet investigated.

The aim of this study to assess C. albicans adherence to conventional heat polymerized acrylic and Valplast denture base materials with considering the effect of saliva coating.

MATERIALS AND METHODS

Specimen preparation

A total of 28 square (10 mm x 10 mm x 3 mm) specimens (14 specimens for each denture base material, acrylic and Valplast) were prepared. Square shaped wax pieces were flasked in a stone. After stone setting and wax elimination, the resulted stone molds were used for the construction of denture materials' specimens according to the manufactures' instructions. For the Valplast, the injection machine type (ZB-A) oven was used for the injection of material capsule. The injection machine was fixed at a temperature of 288ºC and the capsule was grasped by a special holder and placed in a specific hole inside the oven for 16 minutes. Then the material was injected by using a manual press through a hole inside the flask. After 5 minutes the flask was removed from the press and left for bench cooling. The acrylic resin dough was packed into the flask using a hydraulic press. The polymerization process was done by conventional heat curing method (60 minutes at 70 C^0 then at 100 C^0 for 30 minutes). The Valplast and acrylic specimens were left without finishing and polishing then they were immersed in distilled water for 24 hours.

Candida albicans preparation

C. albicans was isolated in a routine smear from patients with denture stomatitis then recultured and identified using gram stain and biochemical tests (germ tube test and C.H.O. fermentation test)[15]. C. albicans was incubated at 37ºC for 24 hours in 500ml sabouraud's broth[16] (Figure1).

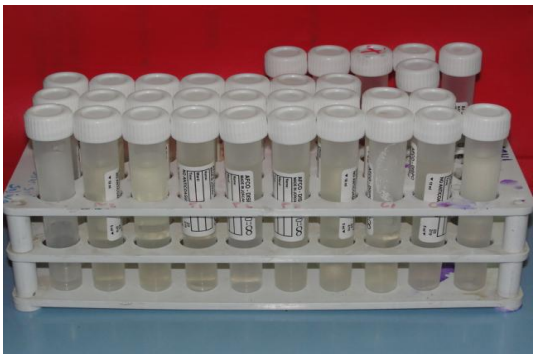

Figure (1): Test tubes containing Sabouraud broth inoculated with C. albicans

And the growth was harvested after 24 hours by cold centrifugation (1700 g/10 minutes) (Figure 2).

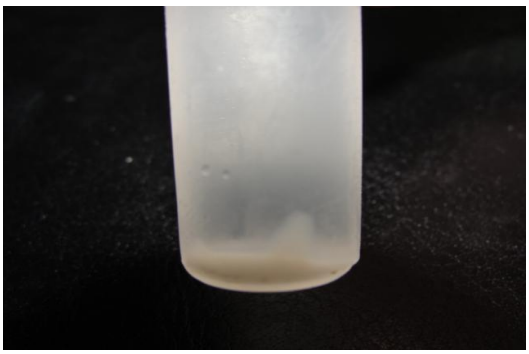

Figure (2): Harvested C. albicans

The resultant pellet was washed twice in phosphate-buffered saline (0.15 mol/L, pH 7.2).[17] Yeast cells were enumerated with a hemocytometer and diluted in phosphate-buffered saline to 10^7 yeast cells/ml.

Adherence assay

The specimens were indepentedly deposited in 20 ml yeast suspension in sterile petridishes and incubated for 1 hour at room temperature. The specimens were washed twice in phosphate buffered saline for 1minute, dried, fixed in methanol 80% and stained for 30 seconds with crystal violet. All materials were examined by light microscopy[4] (Figure 3-6).

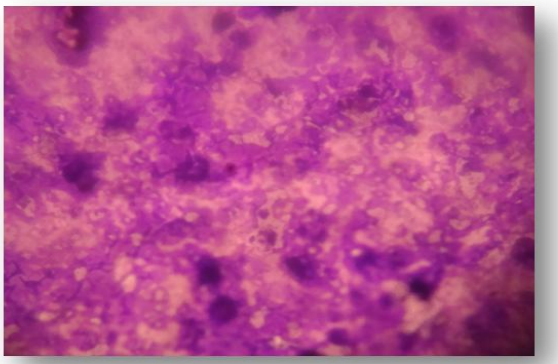

Figure (3): Candida adherence on saliva uncoated acrylic resin specimen

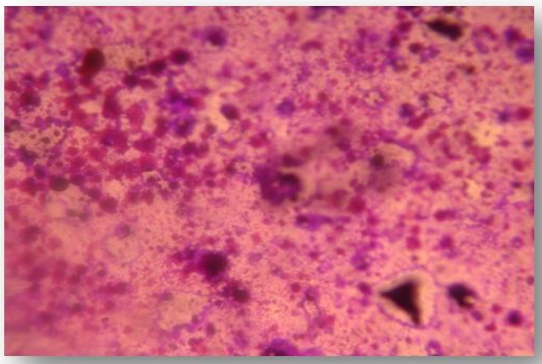

Figure (4): Candida adherence on saliva coated acrylic resin specimen

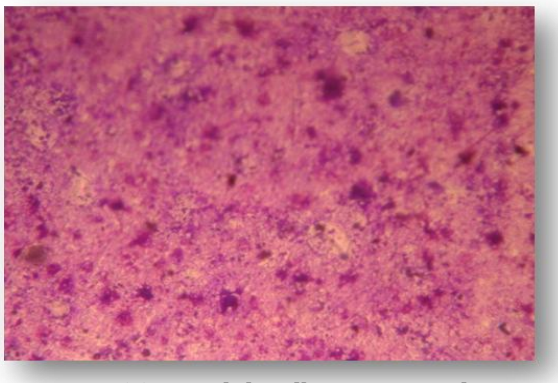

Figure (5): Candida adherence on saliva uncoated Valplast specimen

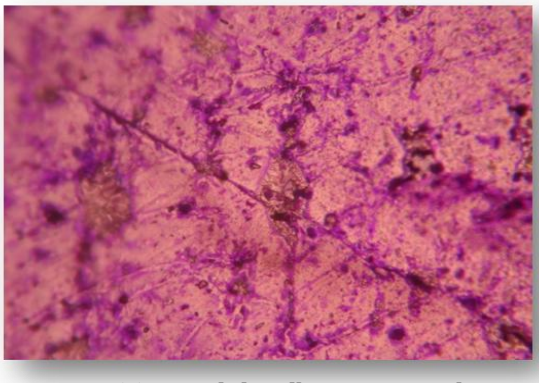

Figure (6): Candida adherence on saliva coated Valplast specimen

Effect of saliva on adherence of C. albicans to denture base materials:

Non stimulated whole saliva was collected from volunteers. Fourteen specimens (7 of each material) were incubated with saliva at room temperature for 30 minutes with gentle agitation, then Candida adherence to the saliva coated materials was determined as previously described.[4]

Candida adherence counts:

Adherent yeast cells in 84 fields of view (0.25 mm^2 per field) in the 28 specimens of materials (3 fields from each specimen) were enumerated and the mean was calculated and the results were expressed as yeast cells per mm^2 of materials.

Statistical analysis :

Means and standard deviations of the adherent candidal counts on saliva- uncoated and saliva- coated specimens were calculated. Simple comparisons were made using unpaired, two tailed t- test between acrylic and Valplast materials and between saliva uncoated and saliva-coated specimens within each material. A *p* value of <0.05 was considered statically significant.

RESULTS

Means of Candida adherence values to the materials were illustrated in Figure (7)

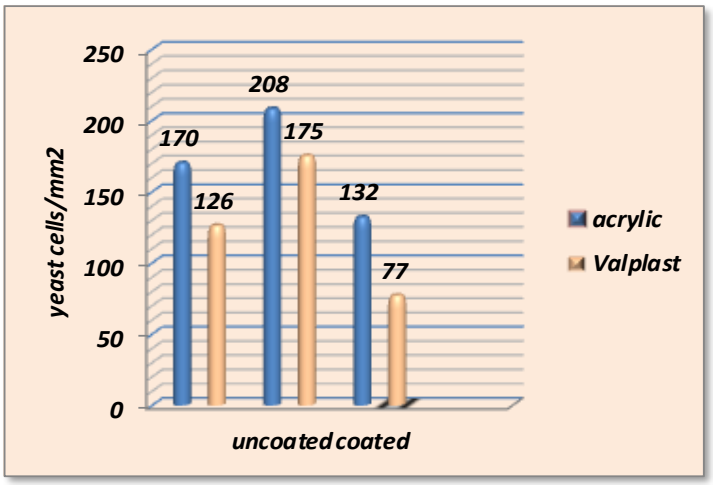

Figure (7): Means of Candida adherence values to different groups

In comparison between the two denture base materials, Candida adherence to acry-lic resin (170 ± 85 yeast cells/mm^2) is gre-ater than Valplast (126 ± 75 yeast cells/ mm^2). Both saliva uncoated and coated acrylic samples have higher means of Candida adherence (208 ± 89, 132 ± 64 cells/ mm^2) than saliva uncoated and coated Valplast samples (175 ± 81, 77 ± 25 cells/ mm^2) respectively. It is clear that the saliva coated materials have lesser Candida adherence than saliva uncoatedones. Table (1).

Table (1): *t–test* of Candida adherence to different groups

Groups	*t–test*	df	Significant Difference
Acrylic-Valplast	2.338	41	0.024
Uncoated acrylic-coated acrylic	4.158	20	0.000
Uncoated Valplast-coated Valplast	5.553	20	0.000
Uncoated acrylic-uncoated Valplast	1.259	20	0.222
Coated acrylic-coated Valplast	3.643	20	0.002

Shows the *t–test* values and the significant difference of the Candida adherence on the tested materials. In comparing the two denture base materials, the difference between the saliva uncoated samples is not significant (p=0.222). Highly significant differences (p<0.001) between saliva uncoated and coated samples of both materials.

DISCUSSION

In this study, a simple *in vitro* model was used to compare the adherence of C. albicans on two denture base materials Vaplast (nylon-based resin) and a commercially available acrylic resin (PMMA-based resin).

The C. albicans species were chosen because it is the most related Candida species to oral fungal infection[18] and the most adherent to polymers which is due to its unique adhesions (CaEap1 cell wall protein) that mediates adhesion with hydrophobic surfaces like polymers.[19] C. albicans has also an important hyphal wall protein 1(Hwp1) which has been found to be required for normal C. albicans biofilm formation.[20] This adhesion offers a unique insight into how C. albicans can survive in the oral cavity.

This research aimed to assess Candida adherence on two materials. Therefore, other variables were kept as constant as possible for both materials like yeast concentration and viability and culture condition. Crystal violet within the study is commonly used in microbiology. It stains all Candida cells present, with no ghost cells evident.

In this study, all the surfaces were prepared in a stone mold and kept without finishing and polishing to further simulate the usual denture fit surfaces which act as a primary reservoir of pathogenesis. This explains why Candida adherence counts on acrylic resin surface differ from those of other researchers who either prepared the sample using a highly polished stainless steel mold,[4] or smoothed and polished the specimens after their construction.[7] While the acrylic results were in agreement with other studies which considered the rough acrylic surfaces prepared in a stone mold.[6]

Acrylic resins have a hidden confounding factor that has antimicrobial properties which is the releasing of residual monomers whereas nylon based resins have none. Despite this fact, saliva uncoated Valplast has the lowest yeasts counts in comparison with acrylic resin. Although the difference is not significant, but it is clear enough to give us an idea about the important effects of different surface roughness, chemical, physical, and hydrophobic properties of different denture base materials on the Candida adhesion. Acrylic resin has rougher surfaces[21] and more porosity[22] than Valplast. Researches demonstrated that material surface roughness and porosity encourage microbial buildup.[6, 23, 24] Further investigations are needed to establish the useful relations of other affecting factors like material surface hydrophobicity and surface free energy on the degree of Candida adherence.

Coating the materials with saliva significantly decreased the number of yeasts/ mm^2. This is in agreement with other *in vitro* and *in vivo* studies regarding denture base and denture lining materials.[4, 5, 7] Saliva acts as a blocker of microbial adhesion to the surface, decreases the surface roughness and surface free energy of resins and this may explain the general decrease of Candida adhesion in those in vitro studies where specimens were coated with saliva.[25]

The Candida adherence on saliva coated acrylic specimens is significantly greater than that of Valplast (p value<0.001). Large cells such as yeasts are more easily dislodged from smooth surfaces than from rough ones. The rough surface of acrylic is known to be a factor in the entrapment of microorganisms and their retention.[6]

CONCLUSIONS

1. C. albicans has lesser opportunities to adhere on Valplast than on acrylic resin denture base materials.
2. Saliva coating of the denture base materials (Valplast and acrylic resin) greatly reduced the Candida adherence.

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